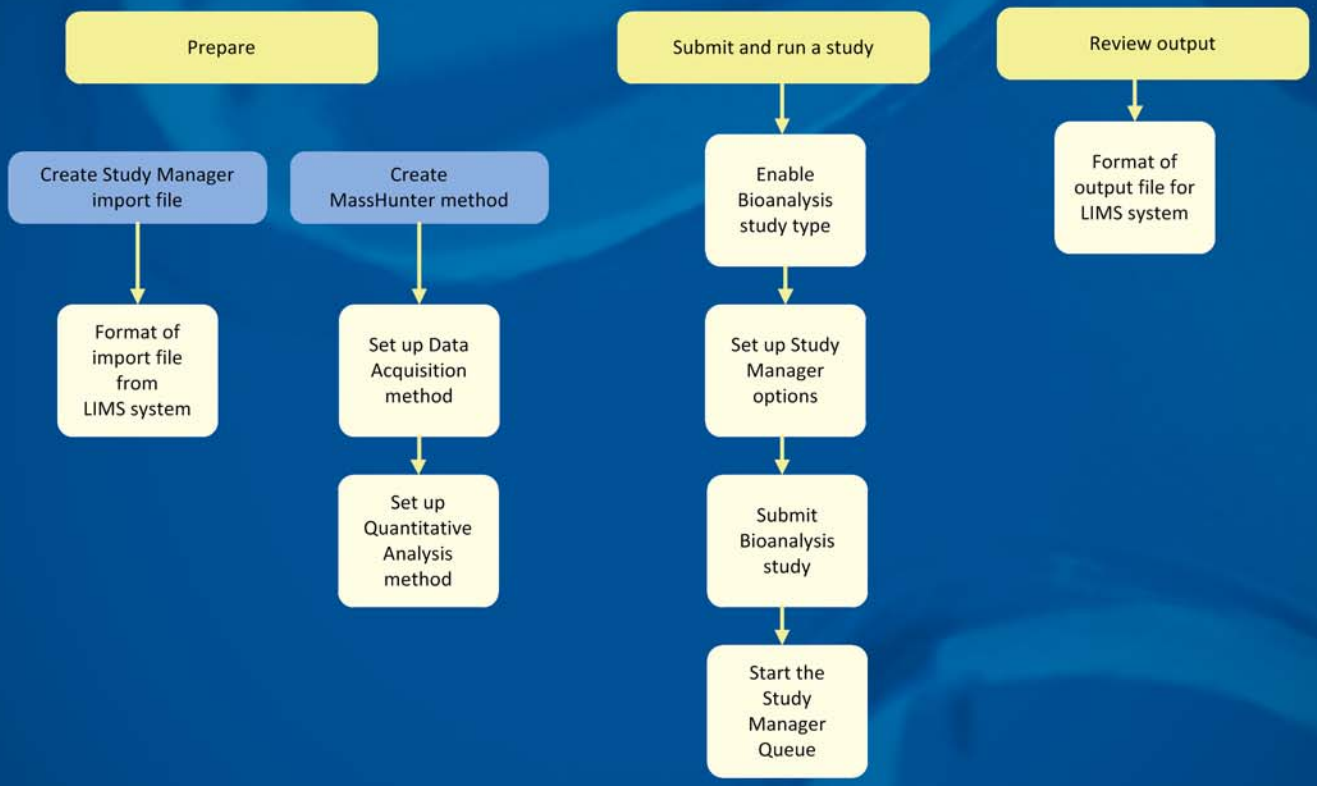
Agilent Bioanalysis

Workflow Overview


Prepare
Create Study Manager import file
Format of import file from LIMS system
Create MassHunter method
Set up Data Acquisition method
Set up Quantitative Analysis method
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Agilent Technologies

Introduction

Bioanalysis is the quantitative measurement of drugs, their metabolites, biological molecules, and biotics in biologial systems. Many samples and replicates are created and need to be quantitated. Automating this process saves time and reduces the number of errors made.

Many laboratories use a Laboratory Information Management System (LIMS) to enter the information for the samples they are analyzing. You create a file in the LIMS system that you import into the Agilent MassHunter Study Manager program to analyze the samples. Bioanalysis encompasses all workflows involving the measuring of the concentration of a drug (and its metabolites) in body fluids over time.

You use the Agilent MassHunter Study Manager program with the Agilent MassHunter Workstation Software - Data Acquisition program to acquire the data and the Agilent MassHunter Workstation Software - Quantitative Analysis program to analyze the data. This overview also describes the input file that you use to create the study in Study Manager and the output file that is created by the MassHunter Quantitative Analysis program. The input file for a Bioanalysis study can be created automatically using a LIMS program. The output file from a Bioanalysis study can be imported back to your LIMS system.

This workflow automates many parts of your analysis. The worklist is created automatically which improves productivity. The number of errors are also reduced because the worklist and quantitative analysis method are created automatically.

Required Items

You use the following Agilent products to run the workflow described in this guide:

- Agilent LC System
  - Agilent 1200 Series LC system
  - Agilent 1260 Infinity Series LC system
  - Agilent 1290 Infinity LC system
- Agilent Mass Spectrometer
  - 6400 Series Triple Quadrupole LC/MS System
- Agilent software to control the instrument and analyze the data:
  - Agilent MassHunter Data Acquisition for QQQ version B.04.00 or higher
  - Agilent MassHunter Quantitative Analysis software version B.04.00 with Service Pack 2 Pharma (B225.19) or higher

Preparing

Before you can submit a Bioanalysis study, you need to prepare the Study Manager import file. You also need to create the MassHunter method. Finally, you also may need to create the Quantitative Analysis method.

Create Study Manager import file

This import file can be created automatically from the LIMS system using the information that you already entered in the LIMS system. The columns in this import file cannot be modified.

The Import File is a text file. Each line in the text file describes a different sample to run. Each field in a line is separated from the next by a Tab. The first line in the file shows a description for each parameter in a line. Some of the parameters are mandatory and must be included. The other parameters are optional or not used.

The different parameters are:

- header=SampleName - optional
- SampleID - optional
- Comments - optional
- AcqMethod- not used
- ProcMethod (the Qualitative Analysis Method)- not used
- RackCode- optional
- PlateCode- optional
- VialPos (Vial Position)- mandatory
- DilutFact (Dilution Factor)- not used
- WeightToVol (Weight to Volume)- optional
- Type (Unknown, Blank, Standard, QC, etc.)
- RackPos (Rack Position)- optional
- PlatePos (Plate Position)- optional
- SetName - not used
- OutputFile- optional
- ~peaknam1
- ~peakcon1
- ~peaknam2
- ~peakcon2

  "~peaknamX" corresponds to either a target or an internal standard (ISTD). It is an ISTD if the corresponding "~peakconX" has a value of 1. The configuration supported are target1, ISTD2, target3, ISTD4 etc where ISTD2 is the ISTD for target1 and ISTD4 is the ISTD for target3.

  The other configuration is target1, target2, target3, ISTD4 where ISTD4 is the ISTD for target1, target2 and target3.

Create MassHunter method

You use the Agilent MassHunter LC/MS Data Acquisition software to set the LC and Triple Quad parameters that you want to use for your analysis.

You probably do not need to use the MassHunter Qualitative Analysis program for this workflow. You can combine the Data Acquisition parameters and the Qualitative Analysis parameters in the same method.

1. Prepare the LC/MS system.

a Condition the analytical column.

b Configure the instrument for data collection.

c Purge the pump.

d Prepare LC mobile phases for your method.

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2. Create the MassHunter Data Acquisition method.

a Enter the LC and MS parameters.

b Save the method.

c (optional) Create the Qualitative Analysis method. This step is usually not needed for a Bioanalysis study.

Create MassHunter Quantitative Analysis method

You can use the Agilent MassHunter Quantitative Analysis program to set up a quantitative analysis method which is used to update a Quantitative Analysis batch. In a Bioanalysis study, you can enter the quantitative analysis information in three different ways:

- Reference Data File - a data file that you already acquired will be used to automatically create the method.
- Quant Method Template - a Quantitative Analysis method that you saved in the Quantitative Analysis program. The calibration levels and concentrations are overwritten by the values from the study.
- Reference Data File by Calibration Level - a data file that will be acquired when this study is run.

The following steps describe how to create the Quant Method Template.

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a Click one of the commands in the **Method > New** menu. Typically, the data file contains MRM data.

b Select the data file that you acquired which is typically a high level calibration sample. Click **OK**.

c Click the **MRM Compound Setup** task in the Method Setup Tasks section in the Method Tasks pane. Review the values.

d Click the **Retention Time Setup** task. Review the values.

e Click the **ISTD Setup** task. Set the ISTD concentrations and specify the ISTD Compound Name.

f Click the **Qualifier Setup** task. Add a qualifier if you want to. Review the values.

In this example, an MRM data file was used to create the method.

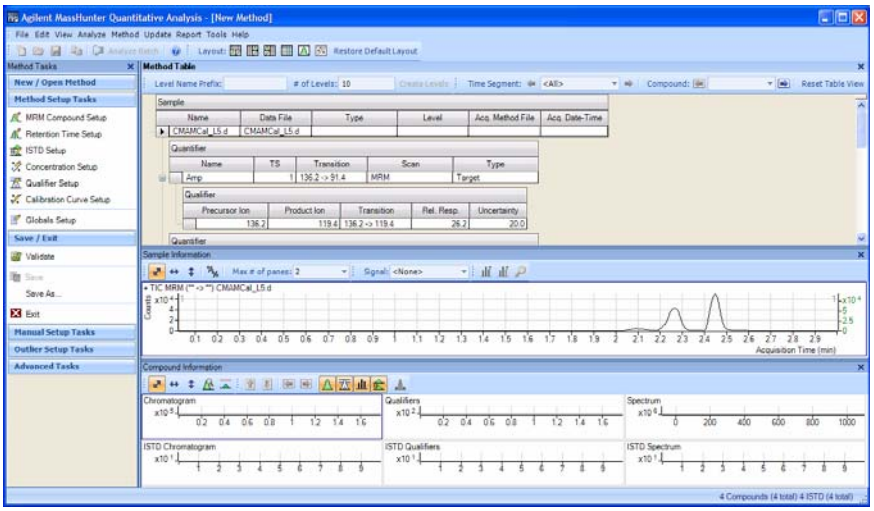

Figure 1 *Method Editor view in the Quantitative Analysis program*

Most of the tasks in the Method Setup Tasks section of the Method Tasks pane are important to do when setting up a new method. The calibration levels for each compound and their corresponding concentrations are different for different studies. The values in this preliminary Quant Method are overwritten with the values from the study. You do not need to enter the calibration levels in this method.

g Validate the method.

h Save the method.

Submitting and Running a Study

1. Enable the Bioanalysis study type

2. Set up Study Manager Options

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a Start the **Study Creator Enabler** program.

b Enable the **Bioanalysis** study.

---

a Start the Study Manager program.

b Set the Study Queue settings on the Settings tab. You can set the following options:

- Whether to pause or continue when an error occurs during a study.
- Whether or not to lock the Data Acquisition program when the Study Queue is running. It is highly recommended that you mark the **Lock Console on QUEUE Start** check box.
- Whether or not to acquire the data files in “locked mode”.
- Whether or not to execute a standby script when the study is idle.

3. Submit the Bioanalysis study and start the Study Manager Queue

a If necessary, start the Study Manager program.

b Submit a **Bioanalysis** study using an input file produced by a LIMS system. You select the Bioanalysis study type in the Select Study Creator dialog box.

In this example, Custom was selected as the second Study Name option. Then, "Bioanalysis" was typed in as the custom name.

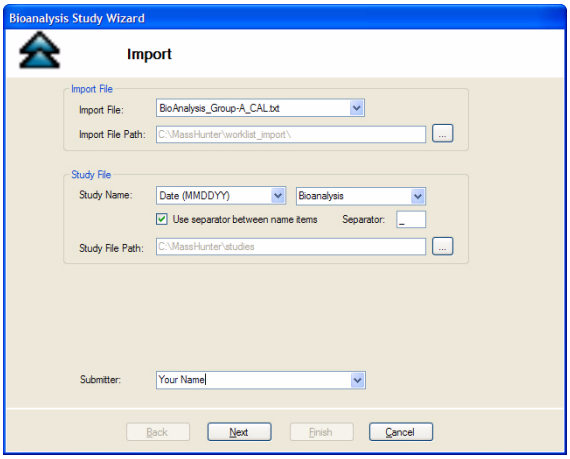
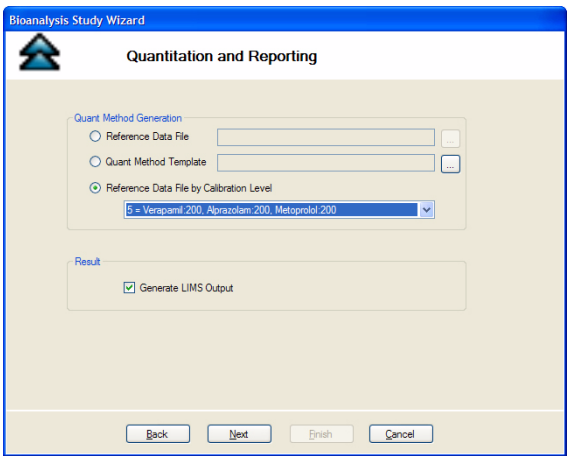


Figure 2 *Bioanalysis Study Wizard - Import page*

In the Bioanalysis Study Wizard - Quantitation and Reporting page, you select one of the options under Quant Method Generation.

- If you click **Reference Data File**, then select the reference data file that you already acquired.
- If you click **Quant Method Template**, then select the **Quant Method Template** that you already created.
- If you click **Reference Data File by Calibration Level**, then select which reference file to use when it is acquired. This data file will be acquired when this study is run.

You mark the **Generate LIMS Output** check box to automatically generate the file that you can import into your LIMS system.



You can click the Quant Method Template button and enter the name of the method that you created in "Create MassHunter Quantitative Analysis method" on page 4.

If you have already acquired the data file, you can also click the Reference Data File button and enter the data file name.

Figure 3 *Bioanalysis Study Wizard - Quantitation and Reporting page*

c Start the Study Manager Queue.

Reviewing Results

If you mark the **Generate LIMS Output** check box on the Bioanalysis Study Wizard - Quantitation and Reporting dialog box (Figure 3 on page 6), then this output file is generated and placed in the study folder. The name of this file is the same as the name of the study with the extension ".txt".

This output file from the Study Manager can be imported directly into the LIMS system. An example of this output file is shown in Figure 4.

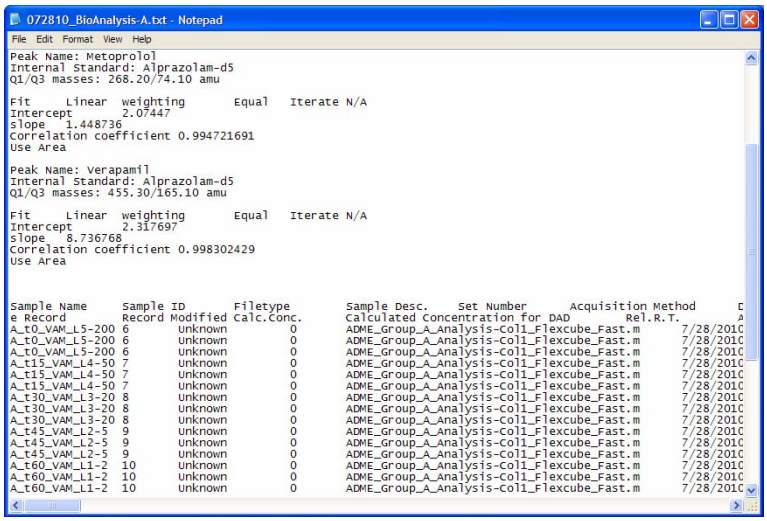

072810_BioAnalysis-A.txt - Notepad
File Edit Format View Help
Peak Name: Metoprolol
Internal Standard: Alprazolam-d5
Q1/Q3 masses: 268.20/74.10 amu
Fit Linear weighting Equal Iterate N/A
Intercept 2.07447
slope 1.448736
Correlation coefficient 0.994721691
Use Area
Peak Name: Verapamil
Internal Standard: Alprazolam-d5
Q1/Q3 masses: 455.30/165.10 amu
Fit Linear weighting Equal Iterate N/A
Intercept 2.317697
slope 8.736768
Correlation coefficient 0.998302429
Use Area
Sample Name Sample ID Filetype Sample Desc. Set Number Acquisition Method
e Record Record Modified Calc.Conc. Calculated Concentration for DAD Rel.R.T.
A_t0_VAM_L5-200 6 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t0_VAM_L5-200 6 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t0_VAM_L5-200 6 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t15_VAM_L4-50 7 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t15_VAM_L4-50 7 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t15_VAM_L4-50 7 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t30_VAM_L3-20 8 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t30_VAM_L3-20 8 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t30_VAM_L3-20 8 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t45_VAM_L2-5 9 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t45_VAM_L2-5 9 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t45_VAM_L2-5 9 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t60_VAM_L1-2 10 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t60_VAM_L1-2 10 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201
A_t60_VAM_L1-2 10 Unknown 0 ADME_Group_A_Analysis-Col1_Flexcube_Fast.m 7/28/201

Figure 4 *The LIMS output file*

For More Information

For details about these procedures, see the *Agilent Bioanalysis Workflow Guide* (Agilent publication 5990-7060EN, Revision A, March 2011).

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